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54 Process for making collagen membranes for medical use.

57 Collagen membranes with desired properties are prepared by using a variety of gel-forming techniques in combination with methods for converting the gels to solid forms. One technique involves compressing a gel matrix under constant pressure to form a fibre network which is dried; another involves disrupting and centrifuging a matrix, harmonizing the precipitate into a paste and casting and drying the paste. The properties of these membranes or other solid forms may be further altered by cross-linking the collagen preparation either after formation of the membrane or gel, or most preferably by mixing cross-linked collagen with solubilized collagen in the original mixture used to create the gel.

EP 0 187 014 A2

**PROCESS FOR MAKING COLLAGEN
MEMBRANES FOR MEDICAL USE**

The invention relates to the field of materials useful for repair of tissue and relevant to wound healing. More precisely, the invention relates to a collagen membranous material prepared by a novel process, which membranes are biocompatible, noninflammatory, and useful in the repair of tissue as artificial implants.

10 Numerous attempts have been made to obtain artificial membranes which can be used as substitutes for skin, blood vessels, ligaments, or other connective tissue. Many of these membranes utilize collagen, as collagen is the major component of connective tissue in
15 general. An extensive literature exists with respect to methods for preparing such membranes, either of collagen alone, (see, for example, US Patent, 4,412,947; Japanese Patent 74/039174; and US Patent 4,242,291) or of collagen in combination with other materials (see, e.g.,
20 U.S. 4,453,939). Other membranes use combinations of materials such as glycoproteins with fibrinogen and thrombin (EPO Application Publication No. 92200, published 26 October 1983), and a combination of keratin derived and glucosaminoglycan polymers (European Patent
25 Publication 89152, published 21 September 1983).

The properties and quality of the resulting membranes with respect to physical characteristics useful in the particular application intended, and their biological characteristics, such as biocompatibility, stability, and integration with surrounding tissue are determined by the nature of the material, e.g., the nature of the collagen used to form the membranes, and on the process used in their formation.

The membranes in the art have had variable success for their intended uses, which include cornea replacements, artificial skin, and wound healing. Many cause inflammation, and have less than optimum properties of flexibility, biological stability, and strength.

The present invention offers a process whereby desirable properties can be obtained in the resulting membrane through the use of nonimmunogenic collagen formed into a membranous material by a spectrum of processes which offer flexibility in the physical properties of the product, so as to permit these properties to be adapted to intended use. The membranous material can be used as a two-dimensional membrane, including membranes which can be shaped to form tubular vessels, shaped into a three-dimensional implant, or formed into a one-dimensional fiber.

25

The invention provides collagen membranes whose physical properties are designed to be suitable for uses in a variety of medical applications including blood vessel repair, uterus repair, reconstruction of lumen surfaces, tendon replacements, and artificial skin. The membranes may also be used as substrates for production of desired cell cultures in vitro. The properties of the membrane are determined by appropriate selection

from a spectrum of preparation processes so as to obtain those characteristics appropriate for a selected use. Similar flexibility is available in the properties of the one- and three-dimensional constructs prepared by
5 modification of, or additions to, the membrane preparation process. The resulting fibers are useful as replacement materials for tendons or ligaments, and may also be used for suturing; the three-dimensional blocks or solids provide implants for use in tissue repair or
10 wound-gap closure.

Thus, in one aspect, the invention relates to collagenous membranous materials which are prepared by the general process of obtaining a gel from a solution of atelopeptide collagen, optionally mixing the solution
15 with a suspension of cross-linked collagen, and converting the gel into membrane form. In another aspect the invention relates to fibers or solids prepared from the gel. In still another aspect, the invention relates to the general process itself, and to
20 the specific methods used within the context of this general process to obtain two-dimensional membranes, fibers, and solids of desired properties.

The gel may be obtained from solubilized collagen or mixture by three alternative approaches. In
25 one approach, the solution of collagen is treated with a precipitating buffer which insolubilizes the collagen by raising the pH. In this approach, both collagen and buffer solutions are precooled almost to freezing, mixed, and then allowed to incubate at approximately
30 room temperature to induce gel formation. In a second approach, the above mixture of collagen and buffer is centrifuged, rather than incubated without gravitational pressure, and the supernatant from the centrifugation is

recovered and allowed to incubate at approximately room temperature. In a third approach, the solution of collagen is treated with an insolubilizing solution at ambient temperature, and the insolubilizing solution is
5 designed to bring the resulting mixture to physiological pH and ionic strength. This mixture is then allowed to incubate at approximately 37°C to create the gel. The third approach may be modified by degassing the mixture immediately upon mixing, and placing the degassed
10 mixture into a mold before incubation.

The procedure in each of the three cases recited above may also be applied to formation of a gel which includes, in addition to the dissolved collagen, a suspension of a cross-linked form. The presence of this
15 additional cross-linked substrate permits further variation in the properties of the membrane material which ultimately results from the method of the invention.

The procedure set forth above for formation of
20 the gel to include the cross-linked collagen in the starting material appears to be critical in the sense that formation of the gel from solubilized collagen alone, followed by partial cross-linking, or cross-linking of the membranes after their formation
25 from the gel, appears to result in a more brittle and unsatisfactory product. Also, if the gel formation procedure from solubilized collagen is modified by the aforementioned addition of cross-linked material, the third method recited is preferred, i.e., the solution of
30 collagen (containing, in admixture, the desired cross-linked material) is treated with an insolubilizing solution at ambient temperature wherein the insolubilizing solution is designed to bring the mixture to physiological pH and ionic strength. The mixture is

allowed to incubate at about 37°C to create the gel. In a particularly preferred embodiment, the mixture is degassed before adding the insolubilizing solution.

The conversion of the gel to a membrane may also be accomplished by two basic alternative approaches. In one approach, the gel is compressed under constant pressure to form a mat which is then dried. Using this method, in addition to obtaining two-dimensional membranes, a solid implant may be prepared directly by compressing the molded gel obtained from the modification of the gel formation process which employs degassing. A fiber product is obtained if the pressure is applied around the circumference of a cylinder formed from the gel. In the second approach, the gel is disrupted, the disrupted gel centrifuged to obtain a precipitate, and the precipitate cast into molds and dried. Depending on the dimensions and shape of the mold, either a membrane or solid can be obtained.

20 Brief Description of the Drawings

Figures 1-2 are electron micrographs at 30,000 x magnification of the prepared membranes G-2, and G-3.

Modes of Carrying Out the Invention

25 A. Preparation of CIS

The process of the invention starts with a collagen in solution, either alone, or in admixture with cross-linked fibrillar collagen. The collagen may be solubilized and purified from mammalian connective tissue, and has been prepared from bovine skin, porcine skin, and mammalian bone, along with a number of other sources. Purification processes are well known in the art. See, for example, U.S. 3,949,073, U.S. 4,066,083, and GB 1,565,340. Collagen can be readily solubilized

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in concentrations useful in the invention by disrupting the fibers in acid, as is well known in the art, and is dissolved at pH values between 1-4. Indeed, collagen in solution (CIS) is commercially available, conveniently, under the trademark Zygen® from Collagen Corporation, Palo Alto, California.

Native collagen exists in a fibrillar form which results from the triple helical structure of the peptide chains. The helical structure is generated by virtue of repeating triplet sequences composed of glycine linked to two amino acids, commonly proline and hydroxyproline in the amino acid sequence. The regions of these triplet repeating units aggregate themselves into triple helical structures. In addition, all collagen chains contain regions at each end which do not have the triplet glycine sequence and are thus not helical. These regions are thought to be responsible for the immunogenicity associated with most collagen preparations, and are called telopeptides. The immunogenicity of a collagen preparation can, in large part, can be mitigated by removal of these telopeptides to produce "atelopeptide collagen". The removal is accomplished by digestion with proteolytic enzymes such as trypsin or pepsin. The nonhelical telopeptide regions are also required to form the cross-links which are responsible for stability of the fibrillar structure in the native material. Atelopeptide collagen must be cross-linked artificially, if it is desired to obtain this characteristic.

The collagen in solution which forms the starting material for the process of the invention is an atelopeptide collagen, preferably a dilute commercially available product such as Zygen® CIS. Concentrations of collagen in the range of 1-10 mg/ml are suitable

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for use in the invention. This range is, of course, suggestive of suitable concentrations and not meant to represent an absolute limitation; any upper and lower limit is arbitrary in this context.

5

B. Preparation of Cross-Linked Collagen

As used herein, "cross-linked collagen" refers to an atelopeptide purified reconstituted collagen preparation which has been artificially cross-linked by chemical or radiation treatment or by other appropriate means to obtain sufficient cross-links that the viscosity of the preparation is 700-3000 centipoise when measured at 22°C and a shear rate of 5000 sec⁻¹. Again, precise limits are arbitrary and this is illustrative of a useful range.

To prepare the cross-linked form, solubilized collagen is first precipitated by neutralizing at room temperature or a preparation of precipitated or reconstituted collagen, such as Zyderm® collagen implant is used, and then cross-linked using standard procedures, including reactivity with chemical cross-linking reagents, such as formaldehyde, glutaraldehyde, glyoxal, and so forth, or with ionizing radiation such as gamma ray radiation. Heat and UV radiation can also be used, but are less efficient. The cross-linked material is then collected by centrifugation and washed with a suitable aqueous solution, such as physiological saline, and the concentration adjusted to a workable level in suspension of 1-10 mg/ml.

In more detail, the cross-linking agent is a polyfunctional, and more usually bifunctional, compound which is used in concentration to produce a viscous, covalently cross-linked collagen before quenching with

an agent which forms an innocuous, water-soluble adduct with the cross-linking agent. The concentration of the collagen in the suspension during the reaction, the concentration of cross-linking agent, and the duration of the cross-linking reaction are significant, but dependent on the nature of the cross-linking agent. The collagen concentration is typically in the range of 0.1-10 mg/ml, more usually 1-5 mg/ml. Aldehydes are preferred as cross-linking agents, and suitable aldehydes include formaldehyde, glutaraldehyde, acid aldehyde, glyoxal pyruvic aldehyde, and aldehyde starch, but preferably glutaraldehyde. Amines are preferred quenching agents, in particular, glycine. The concentration of glutaraldehyde, if this is the selected cross-linker, is typically about 0.001%-0.05% by weight/volume, and the cross-linking reaction takes place over about one-half hour to one week. The reaction is carried out at about 10°C-35°C before adding the quenching agent in at least stoichiometric proportions with the cross-linking agent, although an excess is preferred. One exemplary cross-linking protocol includes a collagen concentration of 3 mg/ml and 0.01% by weight glutaraldehyde for about 16 hours at 22°C. The cross-linked product is washed to remove unreacted cross-linker, polymers formed by the cross-linker, and the unreacted quenching agent, if quenching is employed. A workable buffer is a sodium phosphate/sodium chloride buffer solution of approximately pH 7.

The washed product may be concentrated by filtration or centrifugation to a suitable protein concentration range, which is typically about 20-50 mg/ml, preferably about 25-40 mg/ml. The washed product should have an aldehyde content of less than about 20

ppm and a viscosity in the range of about 700-3000 cp at 22°C measured by an oscillating disc viscosimeter, which measures dynamic, not steady flow, viscosity (Nametre Company, Model 7.006PBD).

5

C. Formation of the Gel

The process of the invention for forming collagen membranes or related membranous materials, comprises, basically, two steps: the formation of a gel
10 from a collagen in solution, and the conversion of the gel to the membrane or other desired form.

Each of these processes may be performed in a spectrum of temperature, gravitational, and ionic strength conditions, and the intermediate solutions may
15 or may not be degassed, and the resulting product will have properties which vary accordingly. The temperature at which gel formation takes place may be between approximately 4°C and approximately 37°C; the ionic strength may vary between about 0.05 to about
20 physiological ionic strength, and the gravitational field conditions may vary from 1 x g to about 13000 x g. The exemplary processes set forth below typify the extremes of these variables, and it is understood that intermediate cases may also be useful, depending on the
25 nature of the membrane desired. Degassing and molding prior to formation of the gel appears to result in a tougher product, which can be further manipulated to form a fiber, membrane or solid.

Three general approaches may be used, as is
30 outlined above. In the first, CIS is cooled and mixed with precooled buffer at an ionic strength well below physiological, preferably about 0.05, and the mixture incubated at low temperature. In the second, the CIS is mixed with buffer at physiological ionic strength and

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temperature conditions and incubated at physiological temperature. In a third approach, the CIS is treated with buffer as in the first method, but subjected to gravitational pressure before incubating at room temperature.

5 The gel may optionally contain an arbitrary amount of previously cross-linked collagen material which is itself prepared from solubilized collagen. The relative amounts of cross-linked and noncross-linked collagen materials appear to determine the properties of the resulting membranes, as well as do the conditions for formation of the gel and membrane stages. The formation of the gel and the conversion to membranes utilizes the same set of options, whether or not the cross-linked material is included in the original composition.

10 In forming the gel, as the intermediate material to the final membranous product to include the cross-linked fibrillar collagen, the gelling procedure is conducted as outlined above, except that, in addition to the collagen in solution, a portion of the suspension containing the cross-linked form at the above-mentioned suspension concentration levels is mixed with the solubilized collagen. The ratio of the two components, based on the relative weights of the collagen contained in each, is quite broad, depending on the nature of the final product desired. For softer and more flexible compositions, a greater proportion of collagen in solution is used; for tougher membranes as final products, a greater concentration of the cross-linked form is used. For example, if the membrane final product is to be used as an artificial tendon to hold sutures or to form a tubular vessel held together by

such sutures, a cross-linked collagen percentage of about 40-80%, preferably around 50%, is preferred.

5 All compositions of membranes formed wherein the original mixture for gel formation contains both a suspension of cross-linked collagen and collagen in solution are expressed as a percentage by weight of cross-linked collagen to total collagen content. Thus, when a membrane is described as having 10% cross-linked collagen, the original components were supplied such that 10% of the total weight of collagen was contained in a cross-linked collagen suspension and 90% was supplied as soluble collagen in solution. The compositions of the invention may contain up to 90% cross-linked collagen.

15

D. Conversion to the Membrane

The conversion of the gel to a membrane may be effected in two basically different ways: either by compressing the gel to squeeze out liquid to form a more cohesive "mat", followed by drying at roughly atmospheric pressure, for example, in air; or by disrupting the gel matrix, centrifuging the disruptate to recover a precipitated collagen, homogenizing the precipitate into a paste, and casting the paste with a mold.

25

The nature of the properties of the resulting membrane depends greatly on which of these two conversion processes is used; the product of the compression process is flexible, translucent, and smooth, and forms a filmlike material with relatively high tensile strength. The product of disrupting the gel followed by precipitation of the disruptate, is relatively brittle and semitransparent, has a rough surface, and is relatively thick.

30

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Either membrane, however, can be characterized as a random fibrillar network wherein the fibrils are approximately of the diameter 70-300 nanometers, and approximately 0.5-5 μ in length.

- 5 The inclusion of cross-linked collagen does not change these properties, except to toughen the product. The cross-linked material is embedded in the fibrillar network described.

- 10 For the compression process, the gel is squeezed in a suitable piston type device, such as, for example, the apparatus presently used to obtain cakes of tofu. Compression is conducted at approximately room temperature by using the collagen gel in situ in the medium in which it was prepared. The compression is
15 applied using 1.1 - 3 atmospheres pressure, and continued until the volume is approximately less than 5% of the original gel. The resulting flat collagen fiber mat is then dried in air or other appropriate atmosphere at a low temperature (less than about 37°C) to obtain
20 the desired membrane. It is also desirable to wash the remaining salts from the membrane. The washing can be effected by washing with water, and redrying, again at atmospheric pressure, at low temperature.

- 25 The process which utilizes disruption of the gel typically is conducted by mechanically disrupting the matrix, such as with a spatula, followed by centrifugation at approximately 8000-16000 x g, preferably about 13000 x g for about 20-30 minutes to obtain the precipitate. The force of centrifugation is,
30 of course, variable and no definite boundary limitations can be set. The precipitate is then homogenized sufficiently to form a pastelike material, at room temperature, and the paste is cast into a mold and allowed to set in at atmospheric pressure at low

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temperature (below about 37°C). The dried material is then desalted, if desired, by washing in water, and redrying.

Cross-linking of the collagen in the resulting
5 membranous material is optional, but can be effected by
treating the membranous material with glutaraldehyde to
obtain the desired cross-links; however, as set forth
above, this approach may result in a more brittle
product. Procedures for this cross-linking are known in
10 the art. Briefly, in typical procedures, the material
is treated with a solution containing 0.05-1%
glutaraldehyde for 1-16 hours, and then quenched by
addition of a glycine solution to a concentration of
about 0.1-0.4M glycine. The cross-linking solution is
15 then removed by washing.

E. Utility

The resulting materials may be employed in the
soft tissue repair constructions ordinarily utilizing
20 artificial membranes, such as burned skin replacements,
tendon reconstruction, or wound repair. They may also
be shaped into various forms and used in connection with
hard tissue repair. The cast or compressed membranes
may be reformed into three dimensional objects for
25 implantation in replacing sections of bone by rolling
into cylinders, or by stacking and cutting to shape.
The membranes may also be used in their two dimensional
configuration by successively packing the membranes into
a defect, such as a cranial or peridental cavity. In
30 general, onlay-type repair may be done by stacking these
membranes into the cavity.

Three dimensional implants are also obtainable
directly from the gel by compression into an appropriate
mold. In this method of construction, it is preferred
that the mixture containing the CIS and precipitating

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buffer be degassed and molded prior to compression.
(Degassing may be used in the related processes which
result in membranes and fibers, also). The dense
collagen fiber network which is formed by compression of
5 the degassed, molded collagen gel is dried, desalted by
washing, remolded before redrying, and, if desired, aged
at elevated temperature to encourage residual
cross-linking. In addition, fibers can be formed
preferably directly from the gel before compression or
10 disruption. The gel is wrapped in a porous, absorbent
material and squeezed or rolled into the desired
diameter fiber. The disrupted gel may also be used, but
in this event fibers must be formed by casting and
stretching, and the process is more cumbersome, leading
15 to a less desirable product.

Examples

The following examples are intended to
illustrate, but not to limit the invention. The first
20 three examples represent alternative methods of forming
the gel, combined with the compression method for
forming a membrane; examples 4-6 represent similar gel
forming methods, followed by membrane formation using
the disruptate, all starting with CIS alone. Examples 7
25 and 8 illustrate formation of cross-links in the
resulting membranes whether the membranes are formed by
compression or by disruption and precipitate recovery.
Example 9 shows the use of degassed and molded mixtures
in gel formation where the gel is used directly in
30 forming a three dimensional implant. Example 10 shows
the formation of a gel which includes a portion of
cross-linked collagen, and Example 11 illustrates the
conversion of this gel into a membrane. Example 12

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illustrates additional embodiments of membranous material which includes cross-linked collagen.

Example 1

5 90 ml Zygen® (2 mg/ml bovine atelopeptide collagen, in HCl, pH1-4) CIS was cooled to 4°C, and mixed with 10 ml of precooled buffer containing 0.2 M Na_2HPO_4 /0.09 M NaOH. The solution was mixed at 4°C, and incubated at room temperature for about 16-20 hours,
10 i.e., overnight, for convenience. The resulting collagen gel was then placed in a press and compressed using constant pressure of about 1.5 atmospheres to a flat collagen fiber network. The resulting network was dried in air at room temperature, washed with water, and
15 redried in air. The resulting collagen membrane was designated G-1.

Example 2

 90 ml of Zygen® CIS at ambient temperature
20 was mixed with 10 ml of room temperature buffer containing 0.2 M Na_2HPO_4 /1.3 M NaCl/0.09 M NaOH, and the mixture incubated at 37°C overnight. The resulting matrix was converted to a membrane as set forth in Example 1. The resulting membrane, G-2, is a smooth
25 flexible translucent material. An electron micrograph of the fiber structure is shown in Figure 1.

Example 3

 90 ml of Zygen® CIS was cooled to 4°C, and
30 mixed rapidly with 10 ml cold (4°C) buffer containing 0.2 M Na_2HPO_4 /0.09 M NaOH, and transferred immediately to centrifuge bottles. The mixture was centrifuged at 8000 x g for 2 hours at about 20°C, and the supernatant recovered from the bottles. The

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supernate was incubated at 20°C for overnight, resulting in the gel. The gel was converted into the membrane in a manner exactly similar to that set forth Example 1, and designated G-3. An electron micrograph of the fiber structure is shown in Figure 2.

Example 4

90 ml Zygen® CIS and 10 ml insolubilizing buffer were mixed at 4°C, and incubated to form a gel exactly as set forth in Example 1. The gel matrix was broken with a spatula, transferred to a centrifuge bottle, and centrifuged at 13,000 x g for 30 minutes. The resulting precipitate was recovered and homogenized into a paste form. The paste was cast into a mold and dried in air at 37°C, then washed with water and redried in air at 37°C to give the membrane P-1.

Example 5

Zygen® CIS was treated with buffer to form a gel exactly as described in Example 2, and the gel then converted to a membrane using the procedure exactly as set forth in Example 4. The resulting membrane was designated P-2.

Example 6

Zygen® CIS was used to form a gel using the procedure as set forth in Example 3, and the resulting gel converted to a membrane as set forth in Example 4. The resulting membrane was designated P-3.

Example 7

90 ml Zygen® CIS at ambient temperature was mixed with 10 ml buffer which contained 0.2 M Na_2HPO_4 /1.3 M NaCl/0.09 M NaOH, and the mixture

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incubated at 37°C overnight. The resulting gel was compressed as set forth in Example 4, dried, and desalted by washing. The washed membrane was then cross-linked by treating with 0.1% glutaraldehyde dissolved in water at 20°C, and the cross-linked membrane washed and dried at low temperature to obtain membrane XG-2.

Example 8

10 A gel was formed from 90 ml Zygen® CIS as described in Example 7, and the resulting gel broken with a spatula, transferred to a centrifuge bottle, and centrifuged at 13000 x g for 30 minutes. The precipitate was recovered and homogenized to a paste.
15 The paste was cast into a mold and dried in air at 37°C, and the resulting membrane washed with water. The washed membrane was then treated with a 0.1% solution of glutaraldehyde, as set forth in Example 7, and the cross-linked membrane washed and dried in to yield
20 membrane XP-2.

Example 9

The procedure for gel formation as set forth in Example 2 was modified by degassing and molding the pre-gel mixture. Before incubation, the mixture was
25 degassed by reduced pressure and placed in a mold. After incubation at 37° for 16-20 hours, the molded gelatin was compressed at about 1.5 atm to obtain a dense fiber network, which was dried in air at 37° or
30 less. The dried solid was desalted by washing, remolded, dried, and aged at an elevated temperature of about 40°C-100°C to increase residual cross-linking, to give the product designated "preformed G-2".

Example 10A. Preparation of Cross-Linked Collagen

Fibrous collagen was reconstituted from collagen in solution (a 3 mg/ml solution of atelopeptide bovine collagen in dilute aqueous HCl, pH 1-4) by adding 0.02 M disodium phosphate to the solution at 18-20°C to obtain a pH of 7.4, and allowing fibers to form for 1-2 hours. To 160 ml of the resulting fibrous collagen suspension, 1.62 ml of 1% aqueous glutaraldehyde at pH 3 was added. The glutaraldehyde solution was added gradually with stirring to obtain a final concentration of 0.01% glutaraldehyde, and the mixture was allowed to react for 16 hours at room temperature before quenching by the addition of 3 M glycine to 0.2 M. After a 1 hour quench time, the cross-linked collagen was washed 3 times with approximately 100 ml of a buffer which contains 0.02 M disodium phosphate, 0.13 M NaCl, pH 7.4, with centrifuging at 17,000 x g for 5-7 minutes between each wash. The dynamic viscosity of the collagen was measured by an oscillating disc device (Nametre Company, Model 7.006PBD) measured at a shear rate of about 5,000 sec^{-1} , and found to be approximately 700 cp at 22°C. After the final wash and centrifugation, the collagen was resuspended in the above buffer to obtain a protein concentration of about 30 mg/ml.

B. Preparation of the Gel

Ten ml of the suspension of VA was mixed with 80 ml Zygen® CIS and the thoroughly mixed components were degassed under a vacuum. To the degassed mixture was added 10 ml of room temperature buffer containing 0.2 M Na_2PO_4 /1.3 M NaCl/0.09 M NaOH, and the mixture was incubated at 37°C overnight. The resulting gel contains 55.5% cross-linked collagen by weight.

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Example 11

The gel of Example 10 was placed in a press and compressed using constant pressure of about 1.5 atmospheres to obtain a flat collagen fiber network.

- 5 The resulting network was dried in air at room temperature, washed with water, and redried in air. The resulting collagen membrane was designated GX-2.

Example 12

- 10 The cross-linked collagen preparation prepared as in 7A of Example 10 is used in a ratio of 1:8 to collagen in solution (prepared as in Example 1) and subjected to the process of Example 1 to obtain a membrane designated GX-1 or of Example 3 to obtain a
15 membrane designated GX-3. Similarly, PX-1, PX-2, and PX-3 are prepared as set forth in Examples 4, 5, and 6, but using a 1:8 mixture of cross-linked to CIS collagen as the starting material.

- 20 Variations in the percentage of cross-linked collagen in the product are also obtained by varying the proportions of the suspension of Example 10A to the CIS.

25

30

Claims

1. A process for preparing a collagen
5 membranous material which comprises compressing a
collagen gel matrix, which matrix optionally includes up
to 90% by weight of cross-linked collagen, with constant
pressure to form a fiber network, and drying said
network.
- 10 2. The process of claim 1 which further
includes the step of cross-linking the material with
glutaraldehyde.
- 15 3. The process of claim 1 wherein the gel is
formed from collagen in solution mixed with a suspension
of cross-linked collagen to obtain a percentage of
cross-linked collagen of up to 90% by weight.
- 20 4. The process of claim 1 wherein the gel is
formed by:
cooling collagen in solution, optionally mixed
with a suspension of cross-linked collagen to obtain a
percentage of cross-linked collagen of up to 90% by
25 weight, to approximately 4°C,
treating the cooled solution with a buffer
solution precooled to approximately 4°C, to obtain a
mixture with a pH of approximately 7 and an ionic
strength of approximately 0.05, and
30 incubating the mixture at about 20°C for about
16-20 hours.

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5. The process of claim 1 wherein the gel is formed by:

mixing at ambient temperature collagen in solution, optionally mixed with a suspension of cross-linked collagen to obtain a percentage of cross-linked collagen of up to 90% by weight, with sufficient salt/buffer solution to obtain a mixture with a pH of approximately 7 and approximately physiological ionic strength, and incubating the mixture at about 37°C for 16-20 hours.

6. The process of claim 1 wherein the gel is formed by:

precooling collagen in solution, optionally mixed with a suspension of cross-linked collagen to obtain a percentage of cross-linked collagen of up to 90% by weight, to about 4°C, mixing the cooled collagen in solution with a buffer solution, precooled to about 4°C to obtain a mixture with a pH of approximately 7 and ionic strength of about 0.05, and centrifuging the mixture at about 8000 x g-13000 x g for 1-2 hours at about 20°C, immediately after mixing to obtain a supernatant, recovering the supernatant, and incubating the supernatant at about 20°C for 16-20 hours.

7. A process for preparing a collagen membranous material which process comprises: disrupting a collagen gel matrix, centrifuging the disrupted matrix at about 13000 x g for about one half-hour,

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homogenizing the resulting precipitate into a
paste,

casting the paste, and
drying the cast paste at a temperature less
5 than 37°C.

8. The process of claim 7 which further
includes cross-linking the membrane with glutaraldehyde

10 9. The process of claim 7 wherein the gel is
formed from collagen in solution mixed with a suspension
of cross-linked collagen to obtain a percentage of
cross-linked collagen of up to 90% by weight.

15 10. The process of claim 7 wherein the gel is
formed by:

cooling collagen in solution, optionally mixed
with a suspension of cross-linked collagen to obtain a
percentage of cross-linked collagen of up to 90% by
20 weight, to approximately 4°C,

treating the cooled solution with a buffer
solution precooled to approximately 4°C, to obtain a
mixture with a pH of approximately 7 and an ionic
strength of approximately 0.05, and

25 incubating the mixture at about 20°C for about
16-20 hours.

11. The process of claim 7 wherein the gel is
formed by:

30 mixing at ambient temperature collagen in
solution, optionally mixed with a suspension of
cross-linked collagen to obtain a percentage of
cross-linked collagen of up to 90% by weight, with
sufficient salt/buffer solution to obtain a mixture with

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a pH of approximately 7 and approximately physiological ionic strength, and

incubating the mixture at about 37°C for 16-20 hours.

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12. The process of claim 7 wherein the gel is formed by:

precooling collagen in solution, optionally mixed with a suspension of cross-linked collagen to
10 obtain a percentage of cross-linked collagen of up to 90% by weight, to about 4°C,

mixing the cooled collagen in solution with a buffer solution, precooled to about 4°C to obtain a mixture with a pH of approximately 7 and ionic strength
15 of about 0.05, and

centrifuging the mixture at about 8000 x g-13000 x g for 1-2 hours at about 20°C, immediately after mixing to obtain a supernatant.

recovering the supernatant, and
20 incubating the supernatant at about 20°C for 16-20 hours.

13. A membranous collagen material prepared by the process of claim 1.

25

14. A membranous collagen material prepared by the process of claim 3.

15. A membranous collagen material prepared by the process of claim 7.

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16. A membranous collagen material prepared by the process of claim 9.

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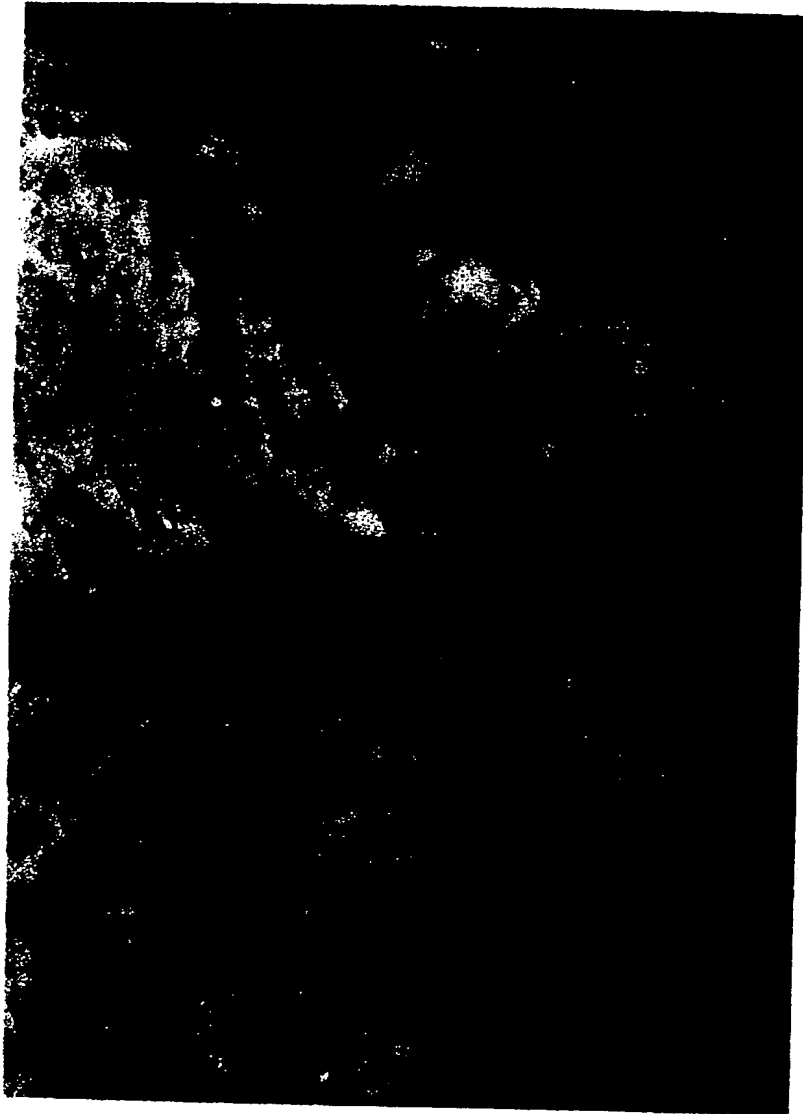


FIG. 1

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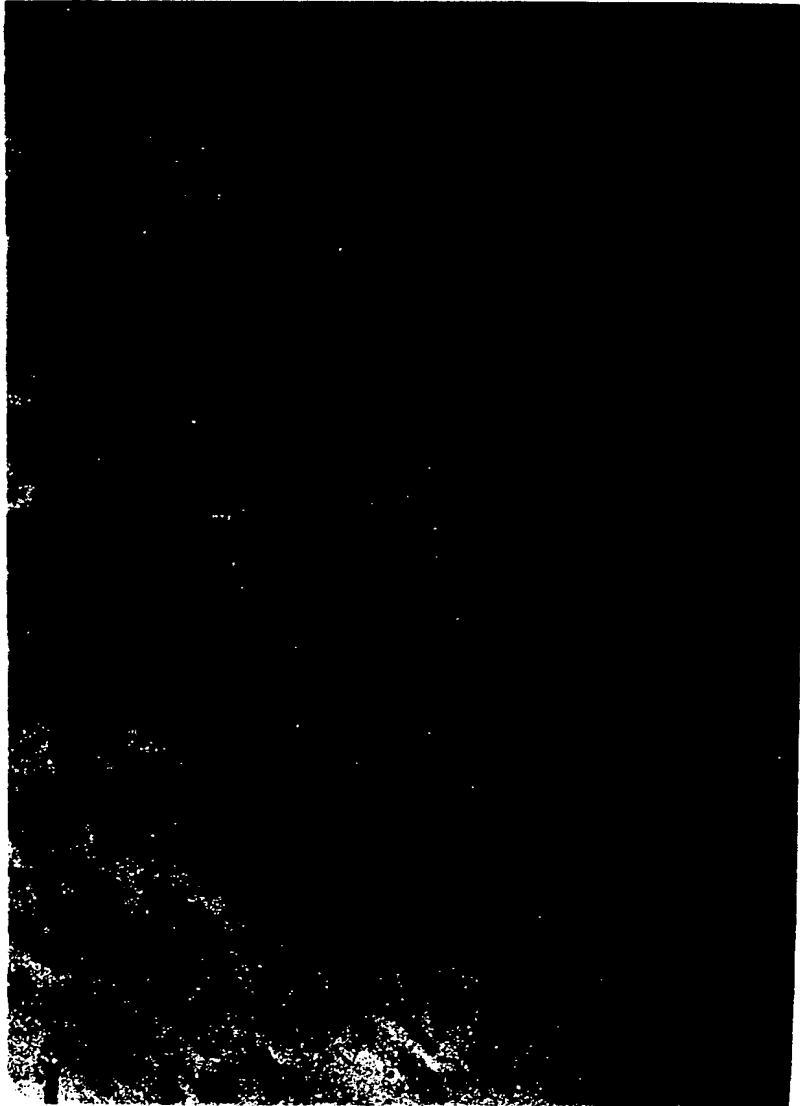


FIG. 2

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